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Antioxidant activity of silver nanoparticles tiwai onion callus extract (*Eleutherine palmifolia* L.Merr)

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ABSTRACT

Silver nanoparticles (AgNPs) are a type of nanotechnology with low chemical reactivity and high stability. AgNPs biosynthesis using plant extracts has advantages compared to other methods because it is simpler and more environmentally friendly. Tiwai onion is a medicinal plant native to Borneo that contains bioactive compounds flavonoid, alkaloid, flavanoid, dan saponin and can be used as a material for producing AgNPs. Onion tiwai calli from tissue culture is an alternative source of material that can be used to avoid excessive use of natural materials. This study aimed to determine the optimum concentration of silver nitrate for the stability of AgNPs and the optimum antioxidant activity of Tiwai onion callus extract and AgNPs. The research design used was a completely randomized design with five treatments, namely, $AgNO_3$ concentrations of 0, 0.5; 1; 1., and 2 mM. This research consisted of tiwai onion callus induction, callus extraction using the maceration method, AgNPs synthesis of onion callus tiwai, and antioxidant activity test using the DPPH method. Temperature, pH, and absorbance data were examined in Microsoft Excel, while antioxidant activity was determined via regression based on IC50 values. The results showed that an $AgNO_3$ concentration of 1 mM was the most stable for forming AgNPs. The highest antioxidant activity was found in the AgNPs of Tiwai onion callus extract, with an IC_{50} value of 48.148 ppm. In conclusion, the antioxidant activity of AgNPs in Tiwai onion callus extract was higher than that of the callus extract.

Keywords: Antioxidants, callus, silver nanoparticles, tiwai onion

INTRODUCTION

Nanotechnology has the potential to advance, because it can be applied to a wide range of scientific subjects. Nanoparticles are an example of nanotechnology (Putra & Yuliar, 2019). According to Abdassah (2017), nanoparticles are extremely small ranging in size from one to 100 nm. Numerous scientific domains, including science, technology, industry, and health, benefit from the use of nanoparticles. Nanotechnology (nanoparticles) has the potential to improve medical diagnostics by improving sensitivity and accuracy, leading to earlier and more precise illness identification (Singh & Amiji, 2021) Nowadays, the most commonly utilized nanoparticles are those made of precious metals, such as silver (Masykuroh & Abna, 2022). Compared to other metals, AgNPs have extremely high stability and low chemical reactivity, making them one of the most researched forms of nanomaterials (Willian et al., 2023). Both physical and chemical techniques can be used to prepare AgNPs. Although clean particles can be produced by the synthesis of nanoparticles using these techniques, they are not environmentally friendly or costly. As a result, a novel technique known as biosynthesis, which involves the production of nanoparticles by biological processes, was developed. This process creates bioreductors using plant-based nanoparticles (Rahim et al., 2020).

Plant extracts, including secondary metabolites, were used to synthesize nanoparticles. Alkaloids, tannins, steroids, phenolics, saponins, and flavonoids are examples of secondary metabolites that can be useful

because they convert silver ions to silver atoms and create silver nanoparticles (Rahmayani et al., 2019). Tiwai onions are among the plants that can be used. Tiwai onions contain a wide variety of secondary metabolite chemicals, including alkaloids, glycosides, flavonoids, phenolics, and steroids. According to Prayitno et al., (2018), tiwai onions have antifungal, antiseptic, antivirial, antimicrobial, antibacterial, and antioxidant properties. Antioxidant compounds are important for human health. Antioxidants can capture, neutralize, and prevent damage caused by free radicals to normal cells (Agustin et al., 2019).

Secondary metabolites are inexhaustible sources of chemicals. Secondary metabolite compounds produced by plants naturally require a very long time and depend on climatic conditions or seasons. Therefore, steps are needed to overcome this, namely, tissue culture techniques (Rohama & Zainuddin, 2021; Sulichantini, 2015). Tissue culture techniques are used to isolate plants from cells, tissues, and organs grown on artificial media under aseptic conditions (Basri, 2016). Some of the advantages of tissue culture are that it can be performed all the time and is not influenced by climate, environment, pests, or seasons. The reproduction system can be regulated, the quality and results are more consistent, and the use of soil (Sulichantini, 2015). In addition, tissue cultures can produce more secondary metabolites in a short time using callus culture techniques (Sutini et al., 2023).

A callus is a collection of undifferentiated amorphous cells formed from tissue cells that divide continuously (Purba et al., 2017). Callus has the ability to reproduce continuously. The cells that make up the callus are parenchymal cells that have a tenuous bond with other cells. Success factors for callus culture include plant growth regulators, medium composition, and explants used (Nurilmala, 2018; Purba et al., 2017). Plant growth regulators are non-nutrient organic compounds that in small amounts can spur, inhibit, and alter the physiological processes of plants (Ernita, 2023). Growth regulators play an important role in growing calli, andthe growth regulators used to grow calli are a combination of auxins and cytokinins (Purba et al., 2017).

Research has been conducted on the biosynthesis of AgNPs using callus extracts. Sari et al. (2023) stated that the best silver nitrate concentration of 1 mM AgNPs using a single garlic callus (*Allium sativum* L.), antioxidant activity test research using ethanol extract of dayak onion bulbus with the best IC50 antioxidant activity value of 41.46 mg/L at a concentration of 82.55% (Mokoginta et al., 2020), and antioxidant activity test research using a single garlic callus with an IC50 value of 33.78 g/ml (Sari & Anwar, 2022). There is no information available on the production of silver nanoparticles and the antioxidant activity of tiwai onion callus, so this study was conducted with the aim of determining the optimal concentration of silver nitrate for the stability of AgNPS in tiwai onion callus extract and the optimal antioxidant activity in callus extract and AgNPS from tiwai onion callus.

METHOD

Tools and materials

This study was conducted from March to August 2024. Induction callus of tiwai onion was carried out at the Tissue Culture Laboratory, Biology Departement. Faculty of Mathematics and Natural Sciences, Mulawarman University. Biosynthesis of silver nanoparticles of tiwai onion callus extract, secondary metabolite test of tiwai onion callus extract, and antioxidant activity test of silver nanoparticles of tiwai onion callus extract will be carried out at the Laboratory of Developmental Physiology and Animal Molecular, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University. The equipment used was a laminar air flow cabinet, analytical balance, stirring hot plate, autoclave, rotary evaporator, UV-Vis spectrophotometer, and an incubator. The plant material was Tiwai onion bulbs obtained from Dayak Market Jl. PM Noor, No.39,

Sempaja Selatan, North Samarinda Subdistrict, 0°27'11.5"S 117°09'51.6"E

Explant sterilization and induction callus

Bulbs are produced in two stages, external and internal. External sterilization of tiwai onion bulbs consisted of washing with detergent for 30 min, soaking the bulbs in a bactericidal fungicide solution with 5 drops of Tween 20 for 30 min, and rinsing the bulbs again using running water for 2 hours (Armila et al., 2014). Deep sterilization was performed in a laminar airflow cabinet (internal sterilization). The tiwai onion bulbs were soaked in 70% alcohol for 1 min, rinsed with sterile distilled water for 3 min, and immersed in 10% clorox which 3 drops of Tween 20 were added for 5 drops for 10 min, after which, the bulbs were rinsed using sterile distilled water three times for 3 min each (Demeke et al., 2014; Sarjani et al., 2023). The last step was to plant the embryos on Murashige-Skoog (MS) media using a growth regulator mixture of picloram (3 mg L -1) and kinetin (1 mg L -1) in a tissue culture environment with 1000–2000 lux of light, at 20–25°C. After three weeks, the calli were subcultured in fresh medium. Callus was subcultured on a medium containing the same plant growth regulator until a large amount of callus is obtained (approximately 100 grams), then extracted as a material for nanoparticle production.

Callus extraction

The solvent used for callus extraction was 95% ethanol, and the extraction process was repeated until the extracted solution became clear (>48 h). The solution was filtered through Whatman paper No. 2 until the filtrate was obtained. The resulting filtrate was evaporated at 50°-55°C where it aims to remove any remaining solvent. The extracted ethanol was then collected. Callus extract (3 g) was dissolved in 30 ml of aquademineralized solution, and the resulting solution was used for nanoparticle biosynthesis (Sari et al., 2023; Botcha & Prattipati, 2020).

Biosynthesis of silver nanoparticles (AgNPs)

The biosynthesis of AgNPs of tiwai onion callus extract can be done by mixing $AgNO_3$ with concentrations of 0.5 mM, 1 mM, 1.5 mM, and 2 mM with tiwai onion callus extract (the ratio of tiwai onion callus extract to $AgNO_3$ is 1:9), Tiwai onion callus extract without the addition of $AgNO_3$ was used as a control. All treatment incubated at 35°C for 8 days, then observed the colloidal color changes of AgNPs before and after mixing (Botcha & Prattipati, 2020).

Stability (temperature, pH) and characterization of AgNPs

Stability tests of AgNPs include measurements of pH (1-10) and temperature (30-100 OC) for each variation in AgNPs concentration. Measurements were taken by dipping a pH meter and thermometer into the nanoparticle solution. The measurement starts from 0-8 days which aimed to determine the stability period of AgNPs from each concentration. AgNPs were characterizedusing UV-Vis spectrophotometry at awavelengths of 350-800 nm (Prasetyo et al., 2021). The AgNPs at the highest concentrations were measured for their antioxidant activity.

Antioxidant activity

The colloidal AgNPs sample was centrifuged for 20 minutes at 4000 rpm and then dried using the freeze-drying process to test for antioxidant activity (Botcha & Prattipati, 2020; Gomatchi et al., 2019). The antioxidant activity test of the best tiwai onion callus extract and AgNPs was carried out using the DPPH (2,2 diphenyl-1-picrylhydrazyl) free radical immersion method (Mokoginta et al., 2020; Syafrizal et al., 2020). In this study, a UV-Vis spectrophotometer with a wavelength of 514 nm was used, which was operated at room temperature wuth little light. The test was performed using the DPPH method with sample concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 ppm. Radical scavenging activity was calculated using ascorbic acid as a control (Arung et al., 2006).

Statistic analysis

Temperature, pH, and absorbance data were examined in Microsoft Excel, while linear regression analysis was used to calculate the inhibitory control value (IC_{50}) of the sample.

RESULTS AND DISCUSSION

Yield extract of tiwai onion callus

The results of the tiwai onion callus extraction carried out by the maceration method using 95% ethanol solvent are shown in Table 1.

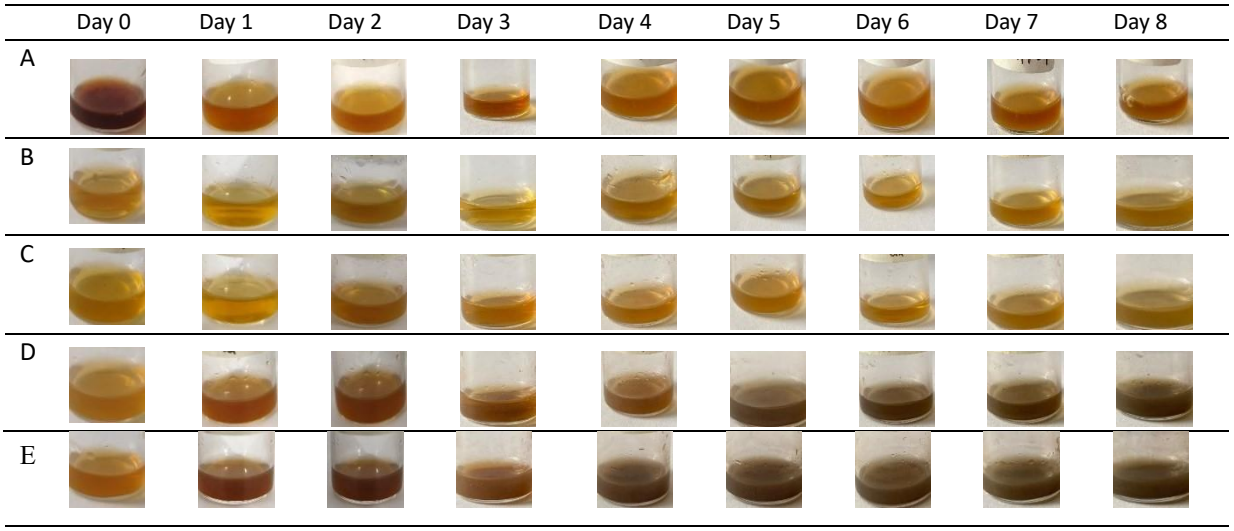
Table 1. Yield Extract of Thiwai Onion Callus.

Weight of Simplisia (g)	Weight of Extract (g)	Yield Weight (%)
104.74	2.92	5.574

The weight of the tiwai onion callus used was 104.74 g and obtained a callus extract of 2.92 g with a yield of 5.574%. From the obtained yield, the metabolite content was quite large. According to Senduk et al. (2020), the yield value obtained from plant extracts is due to the amount of phytochemicals contained in the plant. The more phytochemicals contained in a plant, the higher the yield weight will be.

Many factors affect the weight of the yield, such as the amount of solvent used, type of extraction, and length of time of extraction. Hasnaeni et al. (2019) stated that the process of obtaining the yield weight can be achieved using the maceration method. The maceration method is the simplest extraction method in which the liquid or solvent penetrates the cell wall of the plant and enters the cell cavities containing the active substance, so that the active substance is pushed out of the cell due to the difference in concentration between solutions. In this study, 95% ethanol was used as the solvent. According to Wendersteyt et al. (2021),ethanol is a universal, polar, easily available, selective, and nontoxic solvent. Therefore, this solvent can attract secondary metabolites that are polar, semi-polar, and non-polar in plants. This might cause the yield to be quite large.

Table 2. Color change of biosynthesis AgNPs of tiwai onion callus extract for 8 days of observation (A) control; (B) 0.5 mM $AgNO_3$; (C) 1 mM $AgNO_3$; (D) 1.5 mM $AgNO_3$; (E) 2 mM AgN



Biosynthesis and stability of AgNPs of tiwai onion callus extract

After eight days of treatment $AgNO_3$ concentrations of 0.5; 1; 1.5 and 2 mL of tiwai onion callus extract, the color of the solution changed to reflect AgNPs biosynthesis. At each concentration, the hue of the solution changed from its original bright yellow to brownish yellow, blackish red, and black. One sign that AgNPs form with the bioreductor approach is a change in color. The generated AgNPs are distinguished by a shift in the color of the solution to a brownish yellow hue, and according to Asif et al. (2022), the color shift of the sample indicates

that Ag+ changes to Ag0, which results in the formation of AgNPs. The stability and size of the AgNPs produced throughout the biosynthesis process were indicated by the color change of the sample, according to Badiah et al. (2019). It can be concluded that the nanoparticles generated were stable if the sample turned pale yellow, golden yellow, or yellow-brown. The yellow-brown hue is the most persistent of the three and creates the smallest nanoparticle size. The solution turned brownish yellow at a concentration of 1 mM, which was when the best Tiwai onion callus formed AgNPs. If the sample changes color to black, it indicates that the sample is undergoing an agglomeration process. According to Wibowo et al. (2021), a black precipitate during the biosynthesis of AgNPs indicates that the sample is experiencing agglomeration.

Temperature stability

Temperature changes that occur during the formation of AgNPs in tiwai onion callus extract for 8 days (Figure 1).

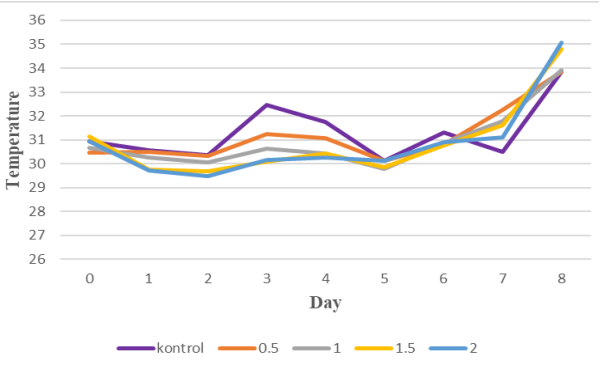


Figure 1. Temperature stability of AgNPs of tiwai onion callus extract.

Temperature changes during AgNPs formation from day 0 to day 8 in all treatments ranged from 29-35C to. The most stable concentration occurred at a concentration of 1 mM because from day to 0-8 it tended to rise significantly at 30^{o}C - 33^{o}C. Temperature stability in the biosynthesis of silver nanoparticles in tiwai onion calli is very important because temperature is an important factor in synthesizing nanoparticles. The temperature can result in the formation of large or small nanoparticles. At the time of nanoparticle formation, heat energy can stimulate secondary metabolite flavonoid, alkaloid, flavanoid, dan saponin electrons in a sample and can reduce Ag^{+} ions to Ag^{0}. In general, nanoparticle biogenesis is carried out at room temperature; however, increasing the temperature from to 30-100°C can expedite the reaction between Ag^{+} and the nucleation of homogenous Ag nuclei, allowing the production of nanoparticles of smaller sizes. Higher temperatures produced smaller nanoparticles.

However, the selection of the incubation temperature in the preparation of nanoparticles must be determined at the optimal temperature that is suitable for the type of sample used (Irwan et al., 2016; Lade & Shanware, 2020; Yanti et al., 2021). Karim et al. (2022) stated that the synthesis of nanoparticles using mangosteen peel yielded the optimal temperature, resulting in the formation of nanoparticles at 30^{o}C.

pH stability

Thr measurement of pH in each treatment in this study obtained results, namely, the increase and decrease in pH in the sample. Changes in pH occurred in each treatment for eight days (Figure 2).

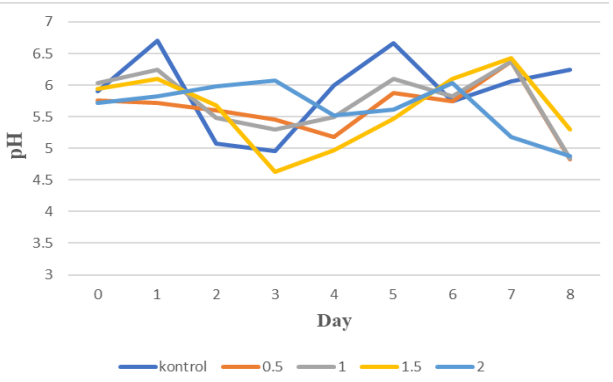


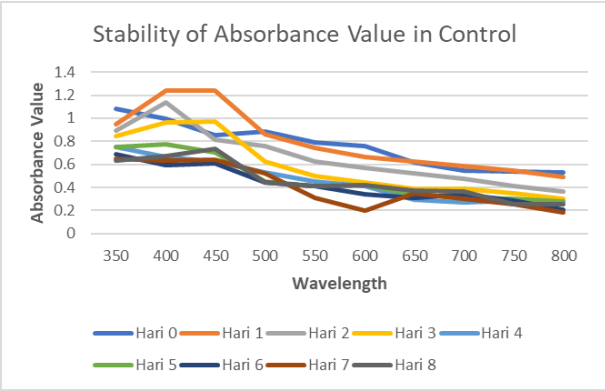
Figure 2. pH stability of AgNPs of tiwai onion callus extract.

Biosynthesis of AgNPs from tiwai onion callus extract showed that the average pH value

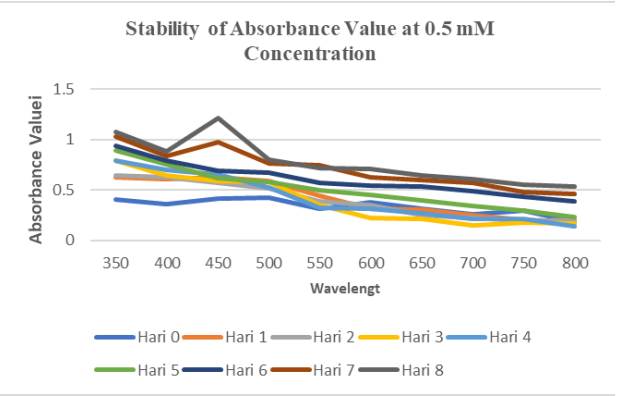
in each treatment from day 0 to day 8 ranged from to 4-6. From each treatment, concentrations of 1 and 2 mM were the most stable concentrations from day 0 to day 8 because there was no significant decrease in pH except on day 8. The concentrations of 0.5 and 1.5 experienced an unstable decrease and increase in pH. pH is also one of the factors that can form nanoparticles because pH has an effect in determining the small or large size of a nanoparticle that is formed. The more acidic the pH in the sample, the larger the nanoparticles formed while the more alkaline the pH in the sample, the smaller the nanoparticles formed. The more alkaline the pH of the sample, the faster the reduction between the Ag mixture and the plant extract. This can be caused by changes in the electrical charge of the biomolecules in the reducer to be more reactive (Qurrataayun et al., 2022). In this study regarding the formation of silver nanoparticles of onion callus extract, the optimal pH for nanoparticle formation was 6, which is in accordance with the research of (Hermansyah et al., 2024). Arif & Noon (2021) stated that the biosynthesis of silver nanoparticles using guava leaves resulted in the most optimal nanoparticle formation at pH 6, which produced nanoparticles measuring 10-14 nm. The changing pH value can be caused by the concentration of $AgNO_3$ and by the effect of the length of incubation time.

Characterization of silver nanoparticles

By monitoring the color shift and determining the maximum wavelength, a UV-Vis spectrophotometer was used to identify whether AgNPs were formed in the sample. Absorbance peaks were used to identify AgNPs at wavelengths betwe en 400 and 450 nm. One of the common wavelengths for the production of AgNPs is this wavelength (Asworo et al., 2023). Figure 3 displays the results of the absorbance stability measurements of AgNPs of tiwai onion callus extract at concentrations of 0.5, 1, 1.5, and 2 mM.



(a)



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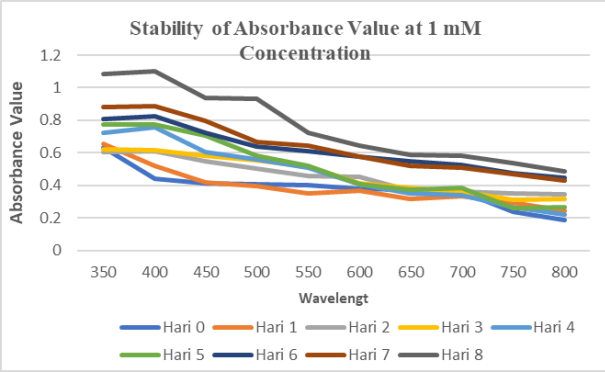

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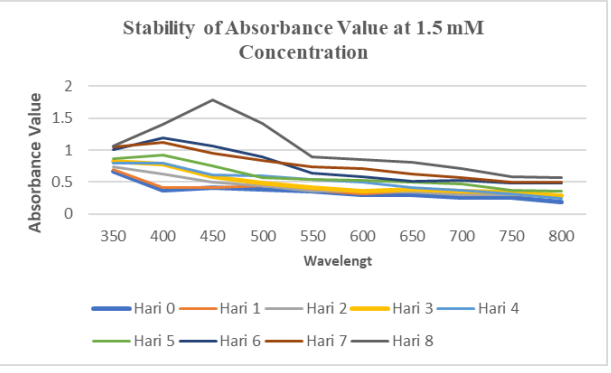
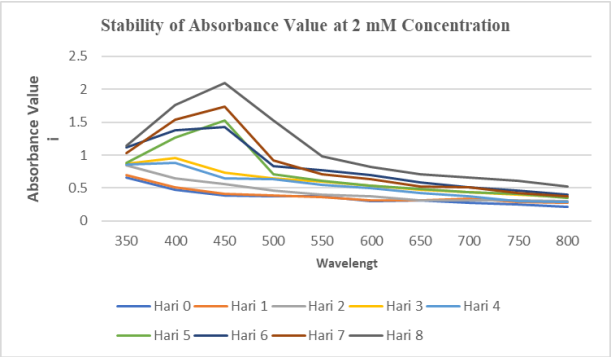


(d)



(e)

Figure 3. UV-VIS spectrophotometer of AgNPs of onion callus extract for 8 days (a) Control, (b) 0.5 mM, (c) 1 mM, (d) 1.5 mM, and (e) 2 mM.

As shown in Figure 3, the absorbance value of AgNPs of tiwai onion callus extract at concentrations of 0.5; 1.5, and 2 mM ranged from to 350-800 nm wavelength, which was carried out for 8 days. Nanoparticles with a concentration of 1 mM are the most stable nanoparticles than other concentrations and have a peak wavelength at 400 nm. This is in accordance with the opinion of Asworo et al. (2023), which states that at wavelength of 400-450 nm is a typical wave of silver nanoparticle formation. The selection of a stable sample minimizes the occurrence of agglomeration. The agglomeration process in the nanoparticle sample increased the size of the nanoparticles.

Incubation time is very influential on the formation of AgNPs the tiwai onion callus extract. The absorbance value graph above shows that as time passed, the peak absorption wavelength or absorbance value of each sample increased. This is because of the formation of nanoparticles, which are becoming increasingly abundant and prone to agglomeration. To avoid this, we must determine the optimal incubation time for the formation of AgNPs. According to Jalab et al. (2021), 48 h is the ideal incubation time for AgNPs.

The sample with a concentration of 1 mM is the best and most stable sample for the creation of AgNPs, as can be observed from the observation of color changes, temperature and pH stability, and absorbance values above. According to Pambudi et al. (2024), the synthesis of silver nanoparticles using spinach thorn leaf extract with an optimal AgNO3 concentration of 1 mM for the formation of silver nanoparticles, and Yanti et al. (2021), the stability of silver nanoparticles to tricarbonate, where the optimal concentration for the formation of silver nanoparticles was 1 mM.

Table 3. Antioxidant activity of tiwai onion callus extract and silver nanoparticles of onion callus extract.

Sample	Concentration (ppm)	Average Absorbance	Inhibition (% Barriers)	IC50 (ppm)
Tiwai Onion Callus Ekstract	3.125	0.325	28.884	71.093
	6.25	0.317	30.634	
	12.5	0.295	35.448	
	25	0.261	42.888	
	50	0.227	50.328	
	100	0.212	53.61	
AgNPs of Tiwai Onion Callus Extract	3.125	0.321	31.991	48.148
	6.25	0.310	34.322	
	12.5	0.266	43.644	
	25	0.233	50.635	
	50	0.208	55.932	
	100	0.192	59.322	

Sample	Concentration (ppm)	Average Absorbance	Inhibition (% Barriers)	IC50 (ppm)
Ascorbic Acid	3.125	0.253	44.638	5.421
	6.25	0.239	47.702	
	12.5	0.170	50.35	
	25	0.169	63.019	
	50	0.166	63.676	
	100	0.145	68.271	

Antioxidant activity

The antioxidant activity of tiwai onion callus extract, AgNPs of Tiwai onion callus extract, and ascorbic acid (as a positive control) in this study are shown in Table 3. Ascorbic acid served as a positive control in this study, which employed the DPPH immersion method. The IC50 (Inhibition Concentration) parameter was used to analyze the test results. There are various categories of antioxidant activity: very strong (IC50 < 50 µg/mL), strong (IC50 50-100 µg/mL), moderate (IC50 101-250 µg/mL), and poor (IC50 251-500 µg/mL). It can be concluded that the sample has relatively low antioxidant activity if the IC50 value is greater than 500. A sample may exhibit very strong antioxidant activity if its IC50 value is low (Souhoka et al., 2019)

Based on the antioxidant activity test table on tiwai onion callus extract with silver nanoparticles of tiwai onion callus extract above, when viewed from the IC50 value between tiwai onion callus extract and silver nanoparticles of tiwai onion callus extract, there is a difference. Based on the IC50 value of tiwai onion callus extract, antioxidant activity was classified as strong, whereas the IC50 value of silver nanoparticles of tiwai onion callus extract was classified as very strong. The antioxidant value contained in the tiwai onion callus extract is lower than the silver nanoparticles of tiwai onion callus extract. This indicates that smaller particle sizes (nanoparticles) will react more easily with DPPH than macro-sized particles, which will be able to increase the antioxidant activity in the sample. This is in accordance with the research of (Nurviana et al., 2020) who demonstrated that the modification of limus seed extract into limus seed extract nanoparticles can increase the content of antioxidant activity, which is indicated by a decrease in the IC50 value from 9.127 to 1.166 ppm.

Modification of plant extracts into nanoparticle extracts can increase their antioxidant activity of the plant extracts. This can occur because the plant extract modified into nanoparticles can increase the surface area of the extract. This results in contact between the antioxidant compounds in the extract and free radicals, which will be greater and more active in reducing their radical effects. The formation of plant extracts into nanoparticles can also increase the stability of antioxidant compounds contained in the extract, which can increase the antioxidant activity (Biswas et al., 2014; Mappamasing et al., 2015).

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the optimum concentration of silver nitrate in the formation of silver nanoparticles of tiwai onion callus extract is at a concentration of 1 mM. Silver nanoparticles of tiwai onion callus extract are formed at a pH close to neutral, which is around 6 with a temperature around 33.92 ^{0}C and is characterized by a change in color to a yellow-brown color. Silver nanoparticles of the tiwai onion callus extract had higher antioxidant activity than the tiwai onion callus extract. More comprehensive characterization testing of silver nanoparticles is recommended, such as Fourier Transform Infra-Red (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM).

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